IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: John Charles Sinclair and Martin Edward Mäntylä Noble

Application No.: 10/530,795 Group: 1656

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For: PROTEIN LATTICE

CERTIFICATE OF MAILING OR TRANSMISSION

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DECLARATION OF JOHN CHARLES SINCLAIR, D.Phil., UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, John Charles Sinclair, of New Biochemistry, South Parks Road, Oxford OX1 3QU, Great Britain, declare and state that:

1. I am a research scientist at the Department of Pharmacology, University of Oxford, Oxford, Great Britain. I received B.A. and D.Phil. degrees from Oxford University in Great Britain. I have extensive scientific experience in all aspects of DNA cloning, protein purification, characterization and crystallization for structural studies of proteins. In the past 10 years, I have performed research and published papers describing the crystal structures of various proteins. Notably, one of my studies was directed to solving the structure of N-acetyltransferase and my work was published in the journal, Nature Structural Biology, in 2000. A copy of my *curriculum vitae* is attached hereto as

"Appendix A."

2. I am one of the two inventors of the subject matter described and claimed in U.S. Patent Application No. 10/530,795, filed November 7, 2005 ("the '795 application").

3. I have studied the Office Action and Interview Summary in the'795 application mailed from the United States Patent and Trademark Office on August 18, 2009 and December 23, 2009, respectively.

4. I have studied the article by Dotan, N., Arad, D., Frolow, F., and Freeman, A., *Angew. Chem. Int. Ed.*, 1999, 38: 2363-2366 (hereinafter, "Dotan") which was cited by the Examiner in the aforementioned Office Action.

5. I state that the individual protein assembly and the resulting lattice constructed from a multitude of the proteins described by Dotan are fundamentally different and lack several key aspects of the invention described in the '795 application. Dotan describes the use of the "nearly tetrahedral lectin concanavalin A," consisting of four (4) peptide subunits (*i.e.*, "monomers") as a building block for the construction of a three-dimensional protein lattice by cross-linking respective subunits of separate individual tetrahedral lectin concanavalin As.

6. Each oligomer assembly shown in Figure 1a of Dotan is lectin concanavalin A (*see* Dotan at page 2364, left col., 3rd paragraph). In Figure 1a, Dotan illustrates two tetrahedral lectin concanavalin As approaching each other in staggered orientation (see the figure legend of Figure 1a of Dotan at page 2364, left col.). There, the respective purple subunits of the two lectin concanavalin As directly juxtapose with each other according to the location of the oligosaccharide binding sites. Each of the oligomer assemblies shown in Figure 1a is not structural equivalent to the "oligomer assembly" described and claimed in the '795 application. The tetrahedral oligomer assembly of Dotan (referred to as the "nearly tetrahedral lectin concanavalin A" by Dotan; see Dotan at page 2364, left col., 3rd paragraph, first sentence) has four (4) subunits (*i.e.*, "tetramer") and indicated as

such in purple, green, red and blue (*see* Dotan, Figure 1a; and Figure 2, the Newman Projections at page 2364). In Dotan, three subunits are generally situated at the bottom and one subunit is situated at the top of the three subunits as a putative vertex of the tetrahedron. Figure 1a of Dotan illustrates a side view of such a tetrahedron. Exhibit B submitted herewith illustrates a top view of the crystal structure of lectin concanavalin A. The binding sites for oligosaccharides are also illustrated at the tip of the tetrahedron in Figure 1a and Exhibit B.

7. In Dotan's tetrahedral lectin concanavalin A, the binding sites for oligosaccharide chains, such as α-D-mannopyranoside or α-D-glucopyranoside, are illustrated at the tip of each tetrahedron vertex. There are a total of four (4) binding sites for the oligosaccharide in one lectin concanavalin A and the binding sites are indicated in white in Figure 1a of Dotan (see Figure 1a of Dotan at page 2364, left col.) and in "ball-and-stick" fashion in Exhibit B. In Dotan's study, the natural binding affinity of the oligosaccharide to lectin was employed as a linker in cross-linking between two respective subunits of two lectin concanavalin As as shown in Figure 1a.

8. Because each lectin concanavalin A has four binding sites, it is connected to four other lectin concanavalin As via cross-links between each of the four (4) subunits and their respective subunits in the other four (4) lectin concanavalin As.

9. In Figure 1a of Dotan and as stated at page 2364, left col. 3rd paragraph, the two tetrahedral lectin concanavalin As are in a staggered position as depicted. Dotan states that: "Cross-linking of 1 by a bismannoside with an appropriate spacer imposing staggered positioning (Figure 1a) *will lead to the formation of the computer-modeled diamondlike three dimension protein lattice shown in Figure 1b*" (Dotan at page 2364, left col., 3rd paragraph; emphasis added).

10. A critical distinction between the construction of the protein lattice described in Dotan and that of the protein lattice described and claimed in the '795 application is that Dotan's tetrahedral lectin concanavalin A has four (4) chiral subunits and does not have a

rotational symmetry axis, whereas the tetrahedral oligomer assemblies described in the '795 application has 12 subunits ("monomers") having tetrahedral *point* groups and at least three rotational symmetry axes. Further, because Dotan's tetrahedral lectin concanavalin A completely lacks a rotational symmetry axis of any kind, it also fails to meet the requirement of axis alignment in the invention described in the '795 application and stated in Claim 1. Even if one assumes that there exists a rotational symmetry in lectin concanavalin A, Dotan clearly states that the lectin concanavalin As are situated in a staggered position with respect to each other in order to achieve a diamondlike supermolecular lattice shown in Figure 1b. It is also noted in Figure 2 that the Newman projections depicting the minimum interaction energy also supports staggered positioning of lectin concanavalin As. Therefore, the rotational symmetries of those oligomer assemblies are not aligned as required in the present invention.

11. I have attached several visual aids as I agreed during the Phone Interview with Examiner Lee held on December 18, 2009, illustrating the critical distinctions mention above. Exhibit A illustrates that the "tetrameric tetrahedral lectin concanavalin A-1" of Dotan does not have any rotational symmetry, whereas the oligomer assembly consisting of twelve (12) subunits (*i.e.*, "monomers") having four (4) tetrahedral point groups as described in the '795 application does. Exhibit B illustrates the top view of the crystal structure of lectin concanavalin A-1 obtain from the Protein Data Bank (http://www.rcsb.org/pdb/explore/explore.do?structureId=3D4K). A true tetrahedron has rotational symmetry axes of order of three (*i.e.*, 120 degree rotation). However, when tetrahedral lectin concanavalin A-1 is rotated around 120 degree from the top view (*i.e.*, one subunit at the top and the other three subunits at the bottom), each individual protein takes on a different orientation, shape and conformation from the original position, failing to achieve any rotational symmetry. In fact, lectin concanavalin A has no rotational symmetry at all due to the *one* chiral subunit ("monomer") situated at the vertex.

12. In contrast, the oligomer assembly consisting of a total of 12 subunits ("monomers") having tetrahedral point groups described in the '795 application has rotational symmetry axes of a true tetrahedron. For example, the '795 application provides *Escherichia coli*

(*E. coli*) dps as an oligomer assembly consisting of 12 subunits having four (4) tetrahedral point groups in which each of the chiral monomers are identical to each other. Exhibit A provides a clear illustration of tetrahedral oligomer assembly of 12 chiral subunits as in *E. coli* dps. Exhibit C illustrates the top view of the crystal structure of *E. coli* dps obtained from the Protein Data Bank (http://www.rcsb.org/pdb/explore/explore.do?structureId=1DPS). As shown in Exhibit A, there are four (4) rotational symmetry axes in the *E. coli* dps oligomer assembly consisting of 12 subunits, each of which extends from the tips or vertex of the tetrahedron. As shown in Exhibits A and C, when rotated 120 degree around any one of the rotational axes at the vertex, the oligomer assembly of the present invention achieves a symmetrical conformation to prior position. Therefore, the oligomer assembly consisting of twelve (12) subunits (*i.e.*, "monomers") and having four (4) tetrahedral point groups do contain a true rotational symmetry of order three (3).

13. The use of an oligomer assembly such as *E. coli* dps allows one to connect the tetrahedron along the true three fold rotational symmetry axis as recited in Claim 1. Therefore, another critical difference between the protein described by Dotan and the oligomer assembly described in the '795 application is that Dotan's protein, which entirely lacks a rotational symmetry axis does not allow one to align one oligomer assembly to another along any rotational symmetry axis as required in the present invention.

14. Dotan discusses the issue regarding the lack of a rotational symmetry at page 2365, left col. 3rd paragraph by explicitly stating that: "The deviation from the true cubic symmetry is apparently due to absence of a true rotational axis in concanavalin A monomers" (Dotan at page 2365, left col. 3rd paragraph). Because there is no true rotational axis in lectin concanavalin A monomer, the entire tetrahedral lectin concanavalin A ("oligomer assembly") consisting of four (4) chiral subunits ("monomer"), one chiral monomer situated at the top of the other three monomers, would also lack a rotational symmetry as demonstrated in Exhibits A and B. Thus, Dotan describes lectin concanavalin A as "nearly" tetrahedral (see Dotan at page 2364, left col., 3rd paragraph, first sentence).

15. In the Interview Summary dated December 28, 2009, the Examiner requested a clarification on the issue regarding Dotan's statement in Section 14 above which was also discussed during the Interview. In the Interview Summary, the Examiner stated that: "The inventor pointed out on page 2365, left column, at the end of 3rd paragraph of Dotan et al., that the concanavalin A monomer does not have a 'true rotational symmetry'. However, the Examiner has pointed out that the presence of a rotational symmetry axis in a monomer was not a limitation of claim 1" (Interview Summary dated December 28, 2009). It is true that the rotational symmetry axis in a monomer is not the requirement of the present invention. However, in the situation where there is a tetrahedral oligomer assembly having only four (4) chiral subunits as in the lectin concanavalin A described in Dotan (*i.e.*, one monomer situated at the top of the other three monomers at the bottom), the lack of rotational symmetry in the chiral monomer situated at the top would inevitably destroy any potential rotational symmetry of the entire tetrahedral oligomer assembly because all the putative rotational symmetry axes of a tetrahedron are at the vertex and they require a rotational symmetry of the molecule(s) situated at the vertex (*i.e.*, "point group"). In contrast to lectin concanavalin A of Dotan, *E. coli* dps of the present invention has three chiral monomers as a point group situated at the vertex and, as a group, they provide a rotational symmetry as demonstrated in Exhibits A and C.

16. The Examiner mistook the subject matter of the present invention and that of Dotan. The protomer of the present invention is a fusion protein containing at least two monomers, each of the monomers derived from an oligomer assembly having requisite rotational symmetry as stated in Claim 1. Thus, the Examiner's statement that Dotan's "protomer comprises at least a first monomer, i.e., a monomeric lectin concanavalin A-1 ("-1" denotes that it is the 1 of 4 tetrameric tetrahedral lectin concanavalin A-1), and a second monomer, i.e., a monomeric lectin concanavalin A-2 ("-2" denotes that it is the 2 of 4 tetrameric tetrahedral lectin concanavalin A-1), fused together,..." is misplaced. It is noted that the two monomers of the putative protomer in Dotan are derived from two separate lectin concanavalin As and each respective monomer is fused/linked with the other via an oligosaccharide chain. The two putative monomers are not derived within

one tetrameric lectin concanavalin A as described in the Office Action. Similarly, the protomer described in the present application has monomers from two separate oligomer assemblies.

17. As discussed in detail above, the oligomer assembly in Dotan (*i.e.*, "lectin concanavalin A") does not have rotational symmetry axes corresponding to a tetrahedral point group. Thus, the following statement by the Examiner's is entirely misplaced.

> "...first tetrameric tetrahedral lectin concanavalin A, which has at least three rotational symmetry axes; and wherein said second monomer is a monomer of a second oligomer assembly, i.e., second tetrameric tetrahedral lectin concanavalin A unit, said second oligomer assembly having a rotational symmetry axis of the same order as one of the at least three rotational symmetry axes of the first oligomer assembly and being aligned with the one of the at least three rotational symmetry axes of the first oligomer assembly when said protomers self-assemble into the lattice." (Office Action at page 23, 1st paragraph)

18. The oligomer assembly in Dotan (*i.e.*, "lectin concanavalin A") is naturally in a "nearly" tetrahedral conformation with four (4) subunits. The four subunits of lectin concanavalin A are held together by its own natural peptide as shown in Exhibit B, not held together by any artificial or designed linker. The crosslinking via an oligosaccharide (*e.g.*, bismannopyranoside 2) described by Dotan is between two respective monomers of two separate lectin concanavalin As, not between the monomers within one lectin concanavalin A (*see* Figure 1a). As such, the following statement by the Examiner regarding Claims 7 and 8 is entirely misplaced: "Claims 7 and 8 are included in this rejection because Dotan et al. teach a linker, i.e., bismannopyranoside 2...which is used to fuse said monomeric lectin concanavalin A in to a tetrameric form" (Office Action at page 23; emphasis added).

19. Given my knowledge of the scientific literature, I state that the protein lattices described in Dotan do not anticipate the protein lattices claimed in the '795 application.

20. I declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements are made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____
John Charles Sinclair, D.Phil.

17th February 2010
Date

Appendix A

Dr. John Charles Sinclair
CURRICULUM VITAE

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Biographical Data

Sex:	Male
Age:	37
Birthdate:	2nd January 1972
Place of Birth:	London, England
Citizenship:	British
Marital Status:	Single

Education

King Edward VI Grammar School, Chelmsford, Essex	1983 – 1990
Lady Margaret Hall, Oxford University	1990 - 1994
Green College, Oxford University	1994 - 1998

Secondary Education

5 O-levels, 3 GCSEs, 3AO-levels	1987/8 All Grade A
4 A-levels (Maths, Chemistry, Physics, Biology)	1990 All Grade A
BA (Chemistry),	1994 Class 2:1
Lady Margaret Hall, University of Oxford	
Dphil	1998

Green College and Department of Pharmacology, University of Oxford
Title: Structural Studies of the Arylamine N-acetyltransferases

Employment and Research

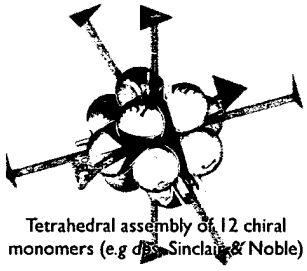
1993 – 1994 Chemistry Part II in the laboratory of Professor Edith Sim, Oxford University Department of Pharmacology. This project involved design, synthesis and assay of inhibitors of the human drug metabolizing enzyme, arylamine N-acetyltransferase.

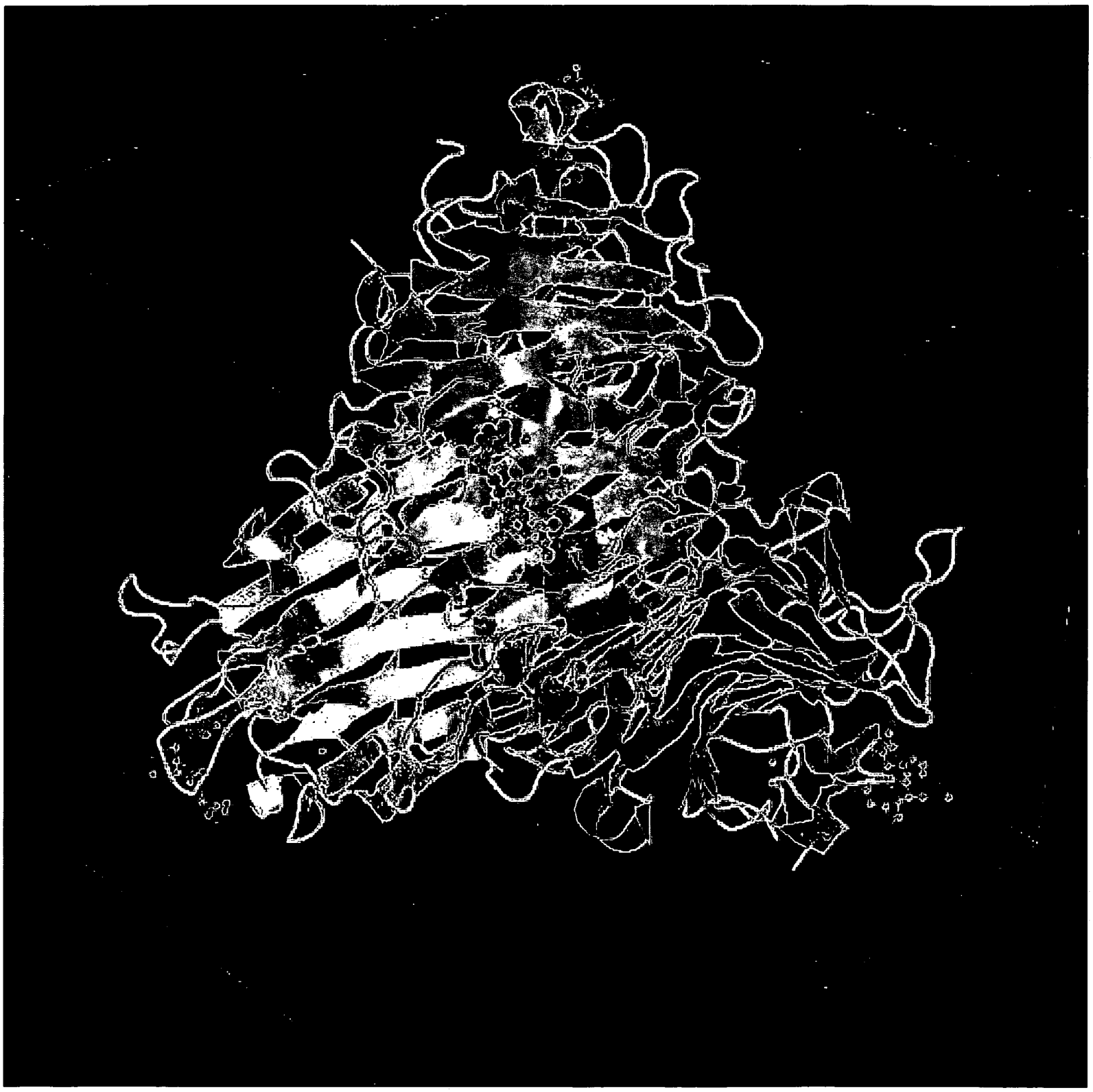
1994 – 1998 DPhil. in the laboratory of Professor Edith Sim. Research involved all aspects of cloning, purification, characterisation and crystallisation of the arylamine N-acetyltransferases for structural study. A paper describing the structure determined as a result of this work was published in Nature Structural Biology.

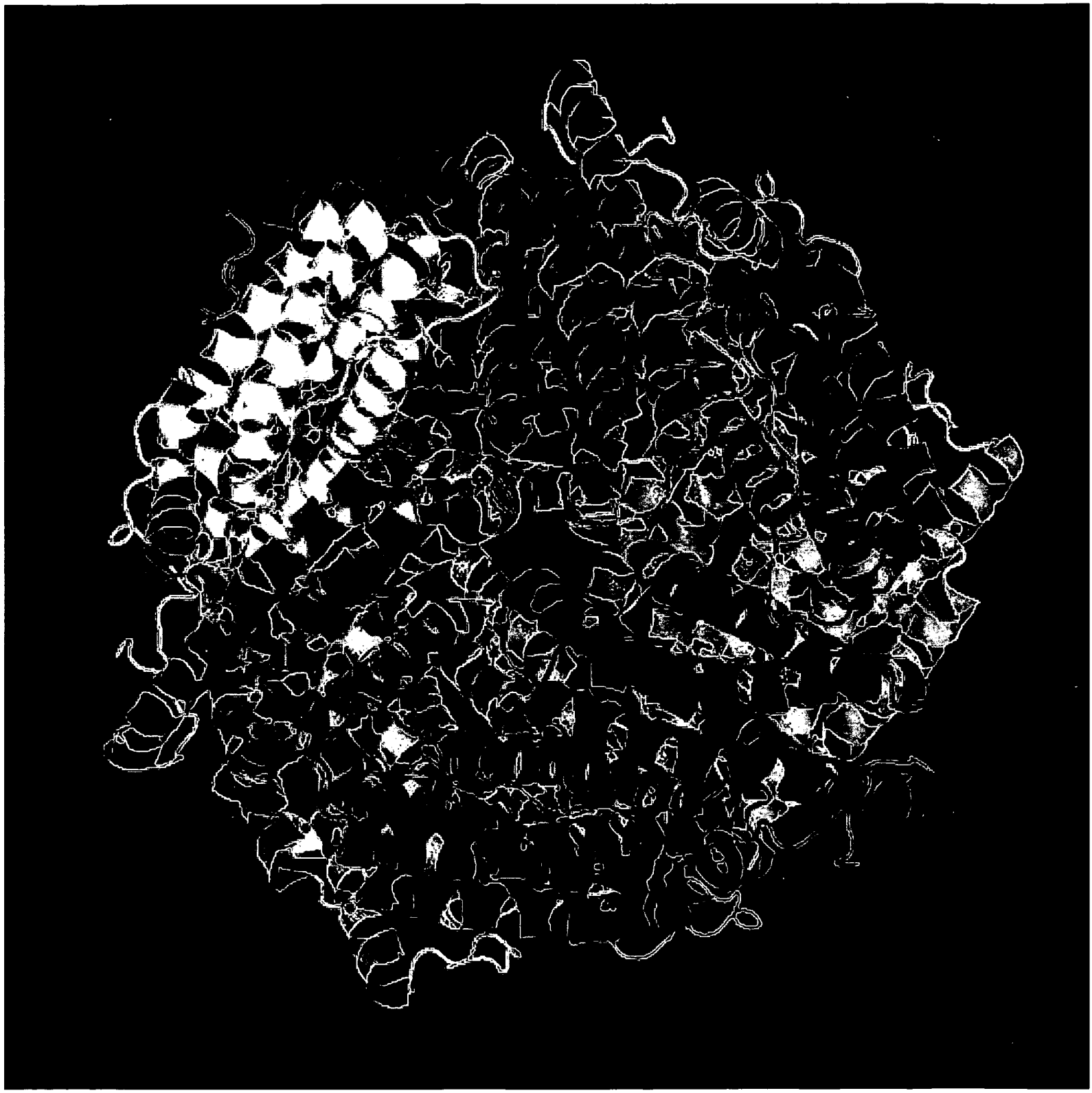
1999 – 2000 Worked with Professor Louise Johnson and Dr. Martin Noble in the Laboratory of Molecular Biophysics, Oxford University towards the determination the structure of the human ZAP-70 kinase. This research was undertaken in collaboration with Roche Products Ltd. and involved cloning, purification and crystallization of the protein from both bacterial and baculoviral expression systems.

2000 – 2003 Development of bacterial, yeast and baculoviral expression systems for a number of cell cycle components. Work was principally focussed on human polo-like kinase (Plk1), human cyclin E and the human cyclin-dependent kinase CDK7 together with its binding partners cyclin H and MAT1. A crystallizable fragment of Plk1 was isolated using expression in baculovirus which was subsequently crystallized after expression in *E.coli*. The structure was solved and published in EMBO journal.

2003 – 2009 Development of self-assembling protein lattices ("crysalins"). The project was co-developed with Professor Martin Noble. Research has formed the basis of two patent applications and a university spin-out, Crysalin Ltd.

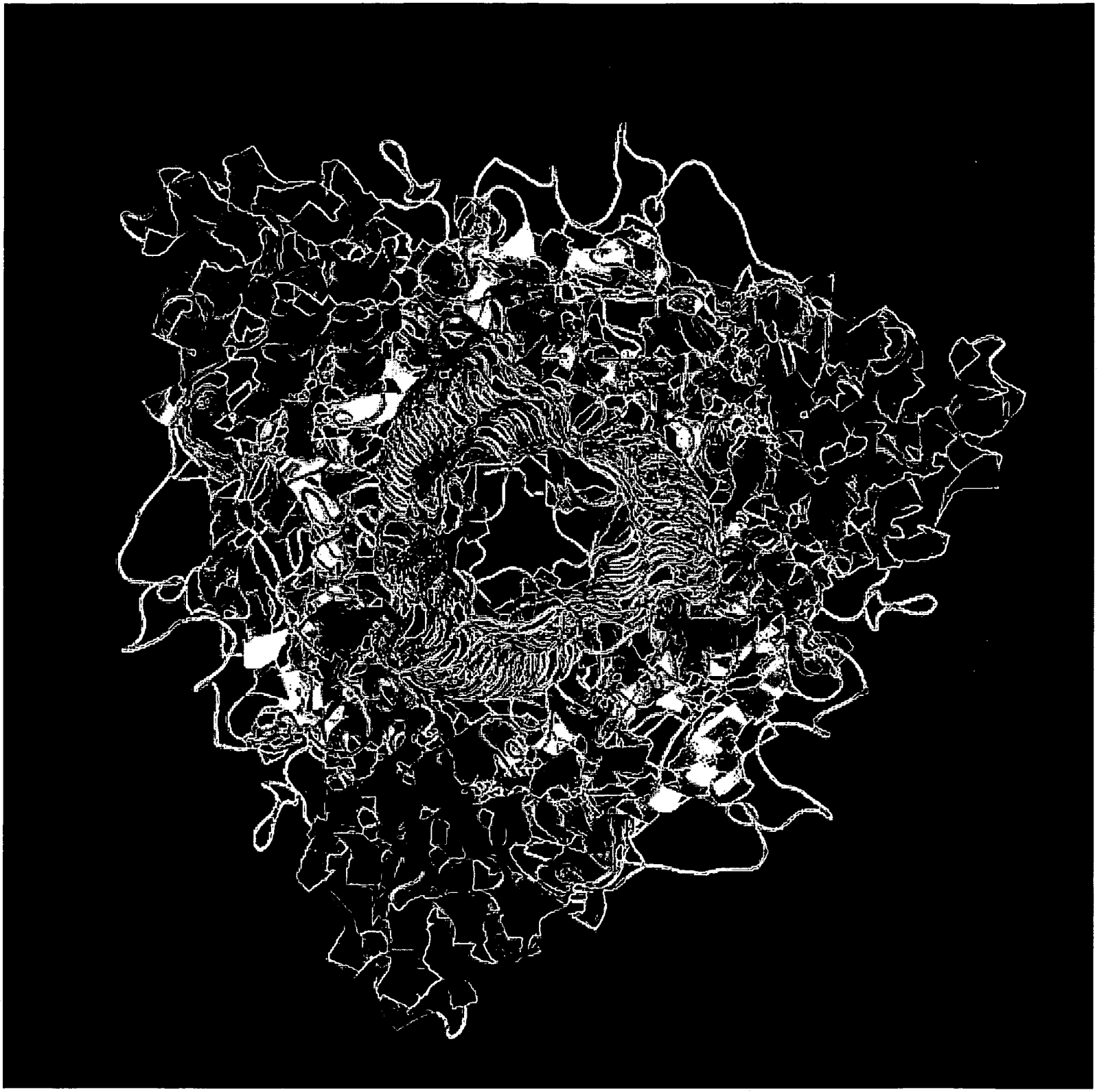
Assembly	Looking into vertex of tetrahedron
 Tetrahedral assembly of 4 chiral monomers (*e.g.* lectin, Dotan *et al.*)	Rotate 120 degrees  Rotate 120 degrees No threefold (or any other) axis of symmetry along which to connect assemblies
 Tetrahedral assembly of 12 chiral monomers (*e.g.* Sinclair & Noble)	Rotate 120 degrees  Rotate 120 degrees Four threefold axes along which the assembly may be connected





EXHIBIT

C



EXHIBIT

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D

EXHIBIT

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E